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Meats: Preservation of Quality by Freezer Storage

INTRODUCTION

Meat is a highly nutritious but extremely perishable food, and its preservation has always been of vital importance to mankind. This is due to the cyclic variation in the abundance of its supply and the necessity to maintain its quality during the periods of shortage and during its distribution and marketing. In order to maintain some degree of palatability and wholesomeness, it must be subjected to some type of processing very soon after slaughter. Freezing and storage at low temperatures are the means by which the highly prized qualities of fresh meat can best be maintained. Similarly, desirable characteristics of special meat products can be frequently maintained satisfactorily.

The full potential of commercial freezing of red meats has not been realized. The meat industry has for some years smoothed out seasonal variations by freezer storage of pork cuts for curing as required. A major area for development, the distribution of frozen cuts at retail levels has made little progress. The reasons for this are manifold and complex. To clear the way for such application, the following must be achieved: marketing reorganization with centralized packaging of retail cuts; adjustment of labor-capital relationships; full acceptance by the consumer of packaged, frozen cuts; and improvement in technology. Meat storage in home freezers and the wide prevalence of locker plants for local patrons have increased during the past several decades. Consumers are well aware of the excellence possible with frozen meat, but the frozen product in many instances cannot compete with the fresh product. This is partly due to a distrust of commercial, frozen meat, the ready availability of fresh cuts, comparative prices, and the relatively poor color of frozen cuts.

QUALITY IN MEAT

Meat is not, on the whole, improved by freezing and storage, but under the proper conditions its quality can be maintained reasonably well. Excellence in meat includes a number of characteristics; these are mainly tender-

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ness, flavor, aroma, juiciness, and color. There is a great deal of interdependence between most of these quality factors. Tenderness is probably the most important single characteristic of meat, because it determines the ease with which it can be chewed and swallowed. Many factors influence the tenderness of meat. These are broadly: species, genetic characteristics, age, degree of finish, antemortem and postmortem treatment, conditioning or ageing, and ultimately the method of cooking used. Tenderness or toughness is a quality representing the summation of properties of the various protein structures of skeletal muscles. The degree of tenderness can be related to three categories of protein in muscle. These are connective tissue, myofibrillar (actin, myosin, and tropomyosin) protein, and sarcoplasmic proteins and reticulum; and the importance of their relative contribution depends on circumstances. Tenderness varies in the muscles of an animal and in a given muscle.

Flavor and aroma are closely related and difficult to define. The flavor of cooked meat arises from water- or fat-soluble precursors. Water extracts of raw meat produce a meaty flavor on heating. Results suggest interaction of meat juices and fibrillar elements during cooking. A purified water extract of raw beef has been found to contain inosine, an inorganic phosphate, and glycoprotein. The glycoprotein, in addition to glucose, contains the amino acids serine, glutamic acid, glycine, alanine, isoleucine, leucine, β -alanine, and proline. Mixtures of these amino acids heated with glucose, inosine, and phosphate produce meaty odors and flavors in fat or water. The opinion has for some time been held by the Japanese that mononucleotides are largely responsible for meat flavor. Glutamic acid, inosine, and hypoxanthine can be used for flavoring. This suggests that the increase in flavor of meat during ageing may be related to nucleotide breakdown, with the formation of inosine, and ribose and hypoxanthine. However, there are reports of artificial production of various meat flavors by heating a pentose with cysteine and other amino acids in water. This suggests that the odor and taste of beef and pork might be simulated by water-soluble constituents. Other research indicates that the components of meat flavor may be divided into two groups. There is a basic meat flavor common to all species which is developed in the lean by heat. The precursors of this can be removed from the muscle tissue with water. Flavors characteristic of species are developed by heating the fatty tissue. There are considerable differences between species in the fatty tissue. However, there may be some doubt that the flavor differences are due basically to variations in unsaturated fatty acid composition. Fat has a definite effect on flavor, and an adequate finish is needed for the best flavor.

Variation in flavor has been noted in sheep and cattle, and there is evidence that this may be inherited. It is well known that older animals have

more flavor than immature animals. There are also notable differences in the flavor of different muscles. Also, the biochemical condition of a muscle may affect its flavor. Muscles with high ultimate pH¹ possibly have lower intensities of flavor.

Juiciness is generally considered in terms of richness and amount of juice. The sensation is influenced by smoothness and a lasting fluidity. This quality characteristic is frequently found to be related to tenderness and flavor. There is a direct association with the amount of intramuscular fat which disappears at higher fat levels. Juiciness is also related to the water-holding capacity of the muscle. Color is important to the overall impression of the quality of the meat. Myoglobin, a respiratory pigment, is mainly responsible for the color, and the appearance of the meat surface is due to the quantity of the myoglobin molecules, its chemical state, and the biochemical condition of the muscle. In fresh meat, the most important chemical form is oxymyoglobin, which has the bright red color desired by the consumer. Poor color may be due to a number of factors, some of which may not be related to poor eating quality. If the ultimate pH is high the color will be dark, due to the presence of the purplish-red myoglobin and a closed structure of the muscle. Low ultimate pH may promote a very pale color due to an open meat structure, and oxidation of the myoglobin to the brown pigment, metmyoglobin. The formation of metmyoglobin frequently foretells general autoxidative deterioration due to coupled reactions with unsaturated fatty acids.

Most of the objective methods of measuring meat quality are inadequate, and determination is largely dependent on sensory methods. However, during the past decade there have been great strides in the basic knowledge of meat science.

THE NATURE OF MEAT

The composition of lean meat may be approximated as 75% water, 18% protein, 4.0% soluble nonprotein substances including mineral components, and 3% fat. The essential unit of muscle tissue is the long multinucleate fiber. The diameters of the muscle fibers vary within the muscle and with the age of the animal and degree of activity. The fiber consists of formed protein elements, the myofibrils, between which is a solution, the sarcoplasm, and a fine network of tubules, the sarcoplasmic reticulum. The fiber is bounded by a very thin membrane, the sarcolemma, to which connective tissue is attached on the outside. Each fiber is composed of many myofibrils, a variable number of nuclei, and inclusions such as mitochondria, glycogen granules, and liposomes or fat droplets embedded in the sar-

¹ "Ultimate pH" is the pH of a tissue after a period of time sufficient for the invariable postmortem increase in tissue acidity to be substantially completed—usually less than 24 hr.

coplastm of the cell. The myofibrillar proteins are myosin, tropomyosin, and actin. These proteins amount to 10% of the muscle and are important in the functional properties of meat. Such proteins undergo changes during rigor mortis that are related to the tenderness and other important properties of muscle. They are the contractile proteins and together with connective tissue constitute the structure of meat. The myofibrillar proteins possess a high degree of the water-holding capacity which is one of meat's most important physical properties.

The sarcoplasmic protein fraction contains most of the enzymatic activities and therefore also influences the functional properties of meat. Myogen, globulins, myoglobin, metmyoglobin, hemoglobin, myoalbumin, creatine kinase, phosphoglyceride dehydrogenase, and pyruvate kinase are components that have been identified in sarcoplasmic extracts. There are said to be at least 50 components, many of which are enzymes of the glycolytic cycle. The remaining structures in meat are the mitochondria containing the insoluble enzymes responsible for respiration and oxidative phosphorylation, the muscle membrane or sarcolemma, and collagen, reticulin, and elastin fibers of the connective tissue. It appears well established that the collagen content has an appreciable effect on tenderness and that the state of the myofibrillar proteins affects both tenderness and waterholding capacity.

Intramuscular fat is an important part of muscle structure, and it influences the characteristics of meat. In addition to triglycerides, there is a considerable content of phospholipids and unsaponifiable constituents such as cholesterol. There are also small but important amounts of vitamins A, B, C, D, E, and K.

There is present in meat about 4.0% of soluble, nonprotein substances. Among these there are nitrogenous compounds such as creatine, inosine monophosphate; di- and triphosphopyridine nucleotides, amino acids, carnosine, and anserine. Carbohydrates are present, including glycogen, glucose, and glucose-6-phosphate. Inorganic constituents such as phosphorus, potassium, sodium, magnesium, calcium, and zinc are concerned mainly with osmotic pressure and electrolyte balance inside and outside the cell. These factors are highly important in muscular contraction and relaxation during life and in postmortem muscle they have a large effect on tenderness and water-holding capacity.

Factors Affecting Meat Characteristics

Breeding.—There is a rough relationship between breed of animal and meat quality. Over years of selection, strains of animals have been developed that tend to yield the best meat. However, a great deal of variability in meat characteristics exists among animals of the same strain. Much of

the selection in the development of strains may have been on the basis of rate of gain, grade, and fat thickness, and not on specific meat quality factors. Tenderness and some aspects of meat flavor have a fairly high degree of heritability. It would seem that much may yet be possible in the improvement of meat quality through effective breeding and selection programs. Excessive fatness in meat animals is a great source of waste today. Intramuscular fat is frequently much higher in the muscles than is necessary for the best quality. Meat cuts destined for freezer storage benefit in stability with a minimum amount of fat. In recent years there has been some improvement in the development of meat-type hogs, and recently more attention is being paid to meatiness in cattle. Intramuscular fat contributes to tenderness, juiciness, and flavor, but does not exert increasing influence beyond a certain modest concentration. Much of the fat added by fast gain is superfluous and wasteful.

Feeding and Management.—Some changes or variation in meat characteristics can be obtained by nutrition and handling. Fatness can be somewhat varied by the level of nutrition. This affects tenderness, juiciness, and possibly flavor. However, in general, lowering the plane of nutrition seems to decrease to a disproportionate degree the marbling or intramuscular fat. This lowers the quality of the meat. In hogs, the use of large amounts of roughage produces a little higher proportion of lean cuts without affecting the intramuscular fat. Feeding provides little leeway in the variation of meat quality. Even use of a low level of nutrition encounters the difficult-to-solve problem of putting the right amount of fat on in the proper location. No single nutrient known will give a consistent and pronounced effect on meat characteristics. The absence or excess of certain substances can cause abnormal characteristics. However, this is a nutritional deficiency condition. Tocopherol or vitamin E, when fed, will tend to increase the stability of fatty tissue. However, this has not been considered to be a practical measure for improving meat. In hogs, the feeding of unsaturated fatty acids causes their deposition in the meat tissue, with the net result of reduced storage life. This may also cause a vitamin E deficiency. Various hormones serve as growth promoting agents. They may increase rate of gain and increase muscle growth with less fat deposition. Tenderness decreases with age. However, flavor increases with age, and also the amount of fat. Differences have been noted between the flavor of grass-fed and dry lot-fed cattle.

Preslaughter History.—Fresh meat of a given species is far from uniform. This is indicated by wide differences in time of keeping in freezer storage. There are inherent biological variations that are responsible for differences in characteristics, quality, and stability of the fresh meat. Also, much lack of uniformity may be due to inability or failure to control condi-

tions in the interval preceding slaughter. Stress exerted on the animals at that time has a considerable physiological effect. Exhaustion, fasting, and excitement tend to cause glycogen loss. However, associated problems range from too much glycogen, with rapid and extensive glycolysis post-mortem, to low glycogen with slow, insufficient glycolysis.

The pig possesses the greatest variation in postmortem changes and differences in muscle characteristics. Pork, not entirely coincidentally, presents the biggest problem of stability in freezer storage. Due to genetic makeup and sensitivity to antemortem conditions, a considerable amount of pork has a rapid glycolysis and is pale and watery with low ultimate pH. The incidence of this condition averages about 18% and may approach 40–50% in the summer. Some strains of hogs are predisposed to this condition, and there may be a genetically controlled excess or imbalance of glycolytic enzymes.

Beef and lamb appear less susceptible to conditions immediately pre-slaughter. However, dark cutting beef is not infrequently encountered. This condition is due to glycogen depletion, with resultant high ultimate pH. There is a considerable difference of opinion concerning the effects of natural and artificial (or induced) glycogen depletion pre-slaughter. At the least, it causes considerable variation in meat properties. Cattle, lambs, and pigs, when subjected to stress conditions for several hours pre-slaughter, usually have lower muscle glycogen, higher postmortem muscle pH, darker color, and improved muscle tenderness and juiciness. Under such conditions, there have been reports of less flavor or decreased desirability in flavor. The nature and extent of this possible (controversial) flavor change is not known. Normal glycogen reserves at slaughter produce meat that is bright in color, low in pH, of an open structure, with minimum water-holding capacity, and greater stability microbiologically.

Postmortem Treatment.—Slaughtering and chilling techniques are fully described in Chapter 6 of Volume 3. Improper procedures can materially affect the quality of the meat and its stability potentialities. With death, the cytochrome oxidase system becomes inactive due to lack of oxygen, and the adenosine triphosphate (ATP) is depleted. The inorganic phosphates formed stimulate the breakdown of glycogen to lactic acid, which lowers the pH. As ATP decreases, actomyosin is formed and rigor mortis sets in. Depending on extent and rapidity of lowering of pH, denaturation of proteins sets in and this lowers the water-holding capacity and causes water loss or drip. Denaturation of the sarcoplasmic proteins makes them more susceptible to proteolysis. Rapid chilling, besides its inhibiting effect on proteolysis and microorganisms, is extremely important for its slowing effect on pH drop, lessening of the amount of denaturation of sarcoplasmic proteins, slowing loss of water-holding capacity, and lessening the shortening

or contraction of the muscles and actomyosin formation. This sequence of events makes a more tender product.

Ageing or Conditioning.—Ageing is a process in which meat is held at various temperatures above freezing. This causes improvement in tenderness and flavor. During this ripening period there is a tendency toward increase in pH and osmotic pressure, proteolysis sets in, and intramolecular rearrangement occurs. Sodium and calcium ions are released from the muscle proteins and potassium ions are absorbed. These changes increase the waterholding capacity. During ageing there is an increase in water-soluble nitrogen which is believed to arise mostly from sarcoplasmic proteins, but proteolysis is not extensive. The degree of protein hydrolysis is less at higher ultimate pH values. There is little known concerning the improvement in flavor usually attained in ageing. The inosinic acid that is found may have a function in the basic meat flavor. However, extended ageing results in loss of flavor. Prolonged ageing is not advisable for meat intended for freezer storage, since much of the stability of the lipids may be lost. It is customary to age beef carcasses. Lamb requires little ageing, and pork, because of its lipid instability, will not tolerate such processing.

FREEZER STORAGE OF MEAT

Freezer storage is a highly effective means of preservation. The main functions are the inhibition of microorganisms and the checking of proteolytic, hydrolytic, and lipolytic activities. Oxidative processes are slowed, but in the case of some products autoxidation may be accelerated during freezer storage.

Drip Upon Thawing

Changes in the muscle protein take place in freezer storage and there is no way of preventing this. Protein denaturation can only be lessened by the freezing technique or by selection of meat with the proper characteristics. One of the most insurmountable disadvantages of freezer stored meat is the exudation of fluid (drip) on thawing. This liquid contains proteins, peptides, amino acids, lactic acid, purines, vitamins of B complex, and various salts. The amount of such constituents is probably related to the degree of cell damage received during freezing and storage. Two factors determine the amount of drip. One controls the extent to which the fluid, once formed, will drain from the meat. Such variables as the size and shape of the cut, ratio of cut surface and amount of large blood vessels are significant. The second factor is related to the water-holding capacity of the muscle proteins. These properties being constant, the amount of drip depends on the rapidity of freezing and the resulting size of the ice crystals. The explanation of this is fairly simple. At very fast rates of freezing, tiny ice crys-

tals are formed in the cells, leaving the meat virtually unchanged structurally. At slow freezing rates, extracellular ice crystals are formed that are quite large. These large crystals distort muscle fibers and damage the sarcolemma. As this proceeds, the remaining extracellular fluid increases in ionic strength and by osmotic pressure is able to draw water from the cell interiors of the muscle. The structure is not only distorted by the large ice crystals, but of far more importance is the denaturation of proteins by the high ionic strength of the extracellular fluid. With denaturation the proteins lose their waterholding capacity. Protein damage is a function of time and temperature of freezing. Thus, the quantity of drip will tend to increase with time in storage. The rapid rate of freezing necessary is frequently impossible to achieve commercially, because of the high thermal inertia of thick cuts. Drip can be minimized by freezing carcasses immediately after slaughter. Ageing before freezing tends to diminish drip to some extent. This is believed due to alterations in ion-protein relationships in which sodium and calcium are released, and potassium ions are absorbed by the myofibrillar proteins. pH has a profound effect on drip. Drip is greater, the lower the pH. At a high ultimate pH, even relatively slow rates of freezing result in virtually no drip. The pH of so-called normal meat is close to the isoelectric point of the proteins. At minimum pH values, the water-holding capacity is low and the proteins are more readily denatured. Even at similar pH's, different muscles vary in amount of drip, and therefore differ in susceptibility to damage. Proteins are far more stable at higher pH's.

Changes in Tenderness and Juiciness

Freezing tends to improve tenderness through a physical action on the tissue. Tenderness appears to hold up well during freezer storage, even though some protein damage occurs progressively. If desiccation is allowed to take place through poor protection, considerable loss in tenderness and juiciness will occur early in storage. Desiccation also favors lipid autoxidation and the development of off-flavors. Even under normal conditions and with adequate protection, there is a tendency toward some loss in juiciness as the result of progressive protein damage. Decrease in water-holding capacity occurs, resulting in loss of fluid (drip) upon thawing, thereby affecting the juiciness of the cooked meat. Loss in water-holding capacity as the result of freezing and subsequent storage is a change that is very difficult to prevent.

Autoxidation of Lipids

The most serious change that takes place in freezer-stored meat is the autoxidation of the lipids. Such deterioration is a problem in all types of

freezer-stored meat and meat products, fresh and cooked meat, cooked and uncooked cured meats, and other meat preparations. Autoxidation of meats is dependent upon availability of and contact with oxygen. Very small amounts of lipid autoxidation are sufficient to produce off-flavors which render the product unpalatable and which may in extreme cases introduce a factor of diminished nutritive properties. The source of most of the off-flavors stems from the oxidative cleavage of the unsaturated fatty acids. A variety of complex reactions occurs and compounds of a volatile and odoriferous nature are formed. The principal class of compounds responsible for rancid flavors and odors seems to be the aldehydes. It is not known whether the basic flavor of meat is affected by the oxidative processes taking place or if the change is due entirely to the added effect of the aldehydes. There are isolated cases where flavor in food has been observed to decrease in the very early stages of autoxidation. However, too little is yet known about meat flavor to definitely determine this influence. Flavors developed by lipid autoxidation can be desirable and characteristic in some special meat products.

The fatty acids in meat are present as part of two different kinds of lipids. These are the triglycerides and the phospholipids. As an integral part of the meat these two lipid classes do not oxidize in a way that is directly related to their unsaturated acid composition. The triglycerides, which are the major lipid, are variable in amount and contain relatively small proportions of polyunsaturated acids. The phospholipids are a fairly constant component of meat. This rather complex and heterogeneous class of compounds contains large proportions of unsaturated acids, and a significant amount of C₂₀ or higher unsaturated acids. Isolated in the pure state the phospholipids oxidize with great rapidity. However, the phospholipids seem quite stable in their natural state in uncooked meat. The triglycerides, either separately or as part of the meat cut, autoxidize readily enough. The initiation of such change or reaction of the triglycerides is either preceded or followed by oxidation of the heme pigment, myoglobin to metmyoglobin. The heme pigments are powerful pro-oxidant catalysts, and it is probable that the ferric pigment, metmyoglobin, accelerates the triglyceride autoxidation. The apparent stability of the very high unsaturated phospholipids at the same time is remarkable since the heme pigments are in virtually the same medium. There appears to be a protective mechanism for the intimately related phospholipids.

The triglycerides, even at low temperature, oxidize readily to form hydroperoxides and their aldehyde scission products. Autoxidation of unsaturated fatty acids has been widely studied. The aldehydes formed from oleate, linoleate, linolenate, and arachidonate have been isolated and identified. Each unsaturated acid forms a characteristic group of aldehydes.

Linoleate and arachidonate are similar in some respects in the products formed.

FREEZER STORAGE OF CURED MEATS

Cured meats autoxidize in the frozen state much more rapidly than uncured or fresh meats. This seeming pro-oxidant effect is due to the presence of sodium chloride, in spite of the fact that salt, except under unusual conditions is apparently not a pro-oxidant. It will not cause the oxidation of methyl linoleate in an emulsion. Nevertheless, in an interface junction between solid sodium chloride and lard, accelerated oxidation has been observed to take place. This observation has been aptly compared to the physical condition existing in frozen cured meat. It has been found that bacon stored at higher levels of freezer storage temperature kept better than bacon stored at 0°F. (−18°C.) or lower. Also, possibly related to this observation is the fact that oxymyoglobin solutions oxidize more rapidly at lower freezer storage temperatures. However, freezer-stored fresh meat does not seem to show such an effect since its stability increases as temperatures are lowered. There may be an indirect relationship involved in this. Sodium chloride has a powerful influence on the protein of meat. It acts on the respiratory enzymes and inhibits most of the meat's native reducing activity. The discoloring effect of sodium chloride on red meat is well known. Upon the addition of salt, the myoglobin is rapidly oxidized to metmyoglobin. The strong action of sodium chloride may set free powerful hematin catalysts, and the ferric are more active than the ferrous heme compounds. In view of these facts, it is not surprising that the fatty acids are exposed to strong oxidative attack. The removal of protective enzyme action and formation of active catalysts by sodium chloride does not explain the tendency toward greater oxidation rates of bacon sides as freezer storage temperatures are lowered. This might well be related to the effect of myoglobin solutions and influence of contact of solid sodium chloride with triglycerides. The effect of freezing on oxidation rates possibly is due to marked changes in pH. Precipitation of ice and salts causes large decreases in the pH of frozen, cured meats. Lowered pH increases the rate of autoxidation of lipids and pigments. The use of nitrite with sodium chloride and heat has been observed to reduce the amount of autoxidation, due to the formation of catalytically inactive ferrous nitric oxide hemochromogen (nitrosohemochromogen). This appears to show that sodium chloride owes its activity to at least two different factors. Increasing the pH or use of an abundant quantity of ascorbic acid will decrease the rate of autoxidation. Cooking of cured meat has been reported to inhibit sodium chloride promoted autoxidation in the freezer. In this case, possibly catalysts are rendered inactive or reducing compounds are formed that protect the triglycerides.

FREEZER STORAGE OF COOKED MEAT PRODUCTS

Cooked fresh or uncured meat and meat products are frequently very unstable. While oxidation is scarcely discernible at the usual freezer storage temperatures, it is extremely rapid during freezing or thawing. There is no induction period and no hydroperoxide accumulation. This deterioration is due to phospholipid oxidation. The mechanism by which the phospholipids are preferentially oxidized is not clear. It is not known whether the sensitivity of the phospholipids is due to heat destruction of protective systems or to the formation of active catalysts. The phospholipids are integral parts of the muscle tissue, and are closely associated with the muscle proteins and pigments. The heat of cooking inactivates enzymes and denatures proteins. Phospholipid oxidation under these conditions is apparently a heme catalyzed reaction. Meats free of heme pigments, such as crab and shrimp, do not show this type of autoxidation. Denatured heme pigments may be more active catalysts. In the case of phospholipid oxidation, acceleration is believed to be brought about by the ferric cooked meat pigment. This deterioration can be prevented by the addition of nitrite, which converts the meat pigments to nitrosomyoglobin and the catalytically inactive ferrous nitric oxide hemochromogen. This indicates that heme compounds are involved; it also shows the catalytic power of the cooked meat pigment. The lipids of meat are therefore composed of two classes which are distributed in the meat tissues differently and appear capable of oxidizing independently. However, the mechanism involved and reasons for the selective action are not clear. The significant action of both salt and heat might be to destroy protective enzyme systems and bring about the formation of more active catalysts through protein denaturation. Sodium chloride apparently causes oxidation by several joint mechanisms. It is not controlled very well by lowering the temperature of freezer storage, and there are no entirely satisfactory antioxidant combinations. The phospholipid oxidation can be controlled by freezer storage and by antioxidants.

THE MICROBIOLOGY OF FROZEN MEAT

So long as meat is held at a temperature lower than 15°F. ($-9^{\circ}\text{C}.$) there is no microbial growth, and all bacteria, yeasts, and molds which may be present are held in a dormant state. At such freezing temperatures, the direct chemical activities of the microorganisms cease and they are unable to produce toxins or enzymes. For a more complete discussion, see Chapter 13. The meat scientist must, however, consider three questions with respect to the microbiology of frozen meat. These are: (1) Can the microorganisms survive freezing? (2) How well can the microorganisms grow after the meat is thawed? and (3) Will extracellular microbial enzymes released before freezing continue to have an effect?

Early workers investigating the survival of microorganisms in frozen meat probably incubated their cultures at 99°F. (37°C.), the temperature usually used in public health laboratories. Counting at this temperature, they noted a decrease in numbers of microorganisms that was correlated with time in freezer storage. When, however, counts are obtained by incubating cultures at 68°F. (20°C.) very little decrease is found with time in the freezer, and where the meat samples are well wrapped to protect the meat from freezer burn and oxidation, there is no significant change in the numbers of microorganisms. Usually, in fact, there will be a slight increase in numbers of microorganisms as compared to the unfrozen controls. This increase is probably only apparent, and due to the effect of freezing in breaking up clumps of organisms on or in the original meat.

Fortunately, however, for our peace of mind, we have also demonstrated that potentially dangerous organisms, such as staphylococci and salmonellae will not grow in ground meat held at 45°F. (7°C.) or below. All refrigerators should, and many do, operate below this temperature.

When we consider our second question, how well can the microorganisms grow after thawing, the facts are even more reassuring. Experimental evidence indicates that microorganisms grow about at the same rate, or even a bit slower, after thawing than they do in unfrozen controls. Furthermore, freezing and thawing results in a prolonged lag phase before the microorganisms begin to grow at all. These studies may be summarized by saying that thawed meat is no more perishable than unfrozen meat.

Many of the microorganisms commonly found on meat excrete extracellular enzymes. These enzymes may be excreted in sufficient quantities prior to freezing to exert a significant effect on meat quality during freezer storage, even though the original microorganisms are completely dormant. The phenomenon has been demonstrated in our laboratory for lipases which are released by *Pseudomonas* cultures and can act on fats at -20°F. (-29°C.) in two weeks. To what extent this may apply to other systems is not yet known, but it emphasizes the fact that strict sanitary precautions must be taken at all stages of the freezing process if high quality is to be assured.

A final precaution should be sounded with respect to precooked, frozen meat dishes. Here the natural spoilage flora have been reduced or completely eliminated by the cooking process and since the food could be contaminated with other microorganisms, such as spores of *Clostridium botulinum* or *C. perfringens*, special care should be exercised to see that the temperature of the food is kept lower than 38°F. (3°C.) prior to cooking. See also Volume 4, Chapter 2.

Packaging

There are a number of means by which deleterious changes of meat in freezer storage may be minimized. Meat cuts should be wrapped in a good type of locker paper with adequate moisture-vapor and air barrier characteristics. A number of different wrapping materials are available, and these are constantly being improved. Good moisture-vapor barrier properties are essential even when long storage is not required. Desiccation or freezer burn can take place rapidly. This results in considerable deterioration in appearance and color. Dehydration in the freezer greatly speeds up the rate of autoxidation, since it causes protein denaturation and allows greater contact and penetration of the air. A locker paper with fairly good barrier properties will permit storage at 0°F. (−18°C.) for 9 to 12 months depending on the kind of meat. Pork will not keep as long as beef and lamb. The ideal method of protection is vacuum or inert gas packing in cans. This type of packaging is so effective that temperature of freezer storage is no longer critical as long as it is no higher than 15°F. (−9°C.). Such storage is expensive and frequently not practical. There are now films available which can be used effectively for vacuum packaging meat. In selecting methods of packaging, consideration should be given to the time in storage required for maintenance of good quality. Oxidation of the fat in meat cuts is largely a surface action and penetration is not deep. The relationship between the surface area and weight of the cut is a factor to be considered in selection of storage conditions.

Antioxidants

There is considerable appropriate interest in the use of antioxidants in meat products. Antioxidants are substances that are capable at low concentrations of slowing the rate of oxidation of lipids or other oxidizable substances. Except for products such as lard or sausage, the use of antioxidants is not approved for meat cuts and meat products. Antioxidants generally used are butylated hydroxyanisole, butylated hydroxytoluene, *n*-propyl gallate, and citric acid. Combinations are used which have effects greater than their performance singly. This behavior is called synergism. Acid compounds, themselves ineffective, have a synergistic action in the presence of phenolic antioxidants and are also able to chelate trace metal autoxidation catalysts. An effective antioxidant for meat cuts would have great advantage for some uses, and it is probable that a suitable one will one day be approved.

The addition of a stabilizing agent to animal tissues constitutes a difficult problem. An antioxidant for meat cuts must meet certain standards. It must

be capable of interrupting the fat-oxygen reaction chain. Its molecules must be small enough to pass readily through animal tissue cell walls. It must have a reasonable solubility range so as to enter both aqueous and fat phases. The stabilizer may not react with the aqueous phase, but if soluble in the aqueous phase the antioxidant will be able to travel through the aqueous channels to reach isolated fatty portions in the heterogeneous product. It must be soluble in an edible medium for dispersion through meat tissue. The antioxidant must have a high inhibitory efficiency so that low concentrations may be used. There must be imparted no flavor, color, odor, or toxicity to the product stabilized and the cost must be reasonable. The mechanism of antioxidant action is not completely understood and there is much leeway for the development of more suitable and effective antioxidants. A number of phenolic antioxidants will retard autoxidation catalyzed by heme pigments. However, the best phenolic inhibitors are practically insoluble in water. In contrast, water-soluble synergistic antioxidants with the exception of ascorbic acid have no effect on heme pigment catalyzed oxidation. Synergistic antioxidants which have been approved for use in lard are citric acid, phosphoric acid, thioldipropionic acid and its esters, and lecithin. Many of the normal constituents of meat, such as amino acids, nicotinic acid, and para-amino benzoic acid, have synergistic activity. The manner in which synergists act is not completely known and probably varies. A number have the property of combining with and inactivating prooxidant trace metals. Some, as in the case of ascorbic acid, may reduce oxidized primary antioxidants. Others, such as phosphoric acid and organic acids, may form fat-soluble complexes with primary phenolic antioxidants. Phosphoric acid will form complexes with fat hydroperoxides.

In meat cuts, color loss due to oxidation of myoglobin is usually coupled with rancidity or lipid autoxidation. Apparently oxidation of one will initiate the autoxidation of the other, but in the normal course of events it is probable that pigment oxidation triggers the process. An antioxidant may be successful in protecting against lipid oxidation but still may not insure color stability. Phenolic antioxidants will not reduce metmyoglobin, and their quinone oxidation products catalyze oxidation of myoglobin. Increased color deterioration due to metmyoglobin formation has been frequently observed in frozen fresh meats treated with phenolic antioxidants. Ascorbic acid under certain conditions will protect meat color. It will reduce metmyoglobin but is not generally suitable, since it accelerates oxidation in some frozen fresh meats and inhibits it in others. Retardation by ascorbic acid is probably dependent on the level of tocopherol present in the meat. The effectiveness of ascorbic acid is increased by the presence of compounds such as ethylenediaminetetra-acetic acid (EDTA) or polyphosphates and sufficient amounts of phenolic antioxidants. However, proper

distribution of phenolic antioxidants in meat cuts is presently impossible. Ascorbic acid combined with liquid smoke has given excellent stability to frozen pork, and it is exceedingly valuable in the fixation and stabilization of color in cured meat.

Aside from difficulties in distribution of antioxidants through meat cuts, antioxidants available generally fall short of being entirely adequate. In the case of uncured, cooked meat, oxidation during freezing and thawing may be controlled by tripolyphosphate, alone or with ascorbate. No satisfactory combination is available for freezer-stored, cured meats. Tripolyphosphate and ascorbic acid will give excellent protection for a time, but then extremely rapid oxidation follows. BHA has proved to be reasonably effective with cured pork, but could not prevent development of off-odor. Frozen, fresh ground meat can be stabilized by any of the approved antioxidants.

Further study on antioxidants is greatly needed. The investigation of naturally occurring materials of potential antioxidant activity appears to be a useful line of research. Among these, the polyhydroxy flavones present in plants are particularly potent and have dual functions or both chain breaking and metal deactivation in the molecule. Many of the flavanoids possess the necessary water solubility because of natural combinations with sugars.

Tocopherol.—The natural antioxidant, tocopherol, in meat is well distributed and reasonably effective but is stored in rather low amounts in animal tissues. If the amount of tocopherol could be increased the problem of lipid stability would be greatly diminished. There have been attempts made at deposition of antioxidants in animal tissues. A large number of antioxidants have been fed to animals but only tocopherols were stored in adipose tissue. A considerable amount of work has been done on the feeding of tocopherol to animals. Increases in body tissues were achieved which gave improved stability. The efficiency of tocopherol storage seemed poor, however, and the process was considered wasteful. This work should be reviewed, and possibly repeated, since much of the evaluation was done on rendered fat and there was no thorough testing of the meat tissues. Tocopherol administration needs further examination in the light of new knowledge of the antioxidant activity of vitamin E, other metabolites, and ubiquinone. Ubiquinone is present in probably much greater concentration in animal tissues than tocopherol. Earlier evaluations of tocopherol feeding were based on the increased content in the rendered fat. Stability cannot be exactly determined without consideration of the tissue as a whole. Much of the pro- and antioxidant activity occurs at interfaces between the fat and the lean. Also, color and phospholipid stability must be considered in addition to the triglycerides.

Smoke, Spices, and MSG.—Some materials added to meat to improve or add distinction to the flavor, also will improve the stability in freezer stor-

age. One of the oldest and most effective is the smoking process. Smoke contains a number of phenols and other classes of compounds. There is much interest in the process of smoking and the composition of smoke, and progress has been made toward identification of the compounds present. Smoke penetration is only superficial, most of it accumulating on the surface. So far as effect on stability is concerned, the problem is as in the case of antioxidants and their dispersion. There are a number of liquid smoke and synthetic smoke preparations available. These formulations vary greatly in their antioxidant properties and contribution to flavor. There are commercial curing mixtures that contain smoke ingredients for the purpose of obtaining uniform distribution through the meat. However, there is little information on the antioxidant properties supplied. Smoking of cured cuts adds greatly to their stability in freezer storage. However, because of the accelerating effect of sodium chloride on rancidity, the freezer storage of cured meats is not generally recommended.

Many of the spices have antioxidant properties, and among them sage is probably the most effective. They are very useful in increasing the stability of freezer-stored pork sausage. However, this is not powerful enough to completely counteract the pro-oxidant action of the sodium chloride.

Monosodium glutamate (MSG) which has the property of intensifying flavors has been known for some time to have properties approaching those of an antioxidant. Experience has indicated considerable stabilizing effect on lipids and color on many types of freezer-stored meat and meat products. The compound not only stabilizes fresh frozen meat cuts and ground meat, but it is strikingly effective with freezer-stored, cured meat and sausage which otherwise deteriorate so fast. Samples treated with glutamate have been found to have greater stability in the freezer than BHA-treated meat. Little appears to have been done to extend the use of glutamate and evaluate it from a basic standpoint. Apparently it penetrates meat tissue fairly rapidly. According to recent research, the compound increases the reducing activity of meat. There appears to be little known about glutamate's capabilities when it is combined with other antioxidants and synergists.

TAILORING MEAT FOR A PURPOSE

As already referred to, fresh meat of any species is not at all uniform in its resistance to oxidative deterioration. Differences in fatty acid and toopherol content are not great enough to account for this extreme biological variation. The history of the meat may one day determine its selection for use as fresh meat, cured, frozen, dehydrated, or canned. One of the most important properties of meat is its ultimate pH. This may vary from about 5.2 to 6.6 due to preslaughter conditions and environment, which were earlier discussed. If the pH is lowered within the range of normal meat, fresh

meat will be discolored, cured meat color will be improved, the oxidation of the fat of raw meat will be accelerated, and the stability of the fat of cooked meat will be unchanged. Variations through this range of pH can have tremendous bearing on the properties of the meat to be freezer-stored. Fresh meat with pH at the upper end of the normal pH scale will show a much more stable frozen product from the standpoint of lipids, color, and waterholding capacity. Also there should be little drip on thawing. Indeed, selection or adjustment of the pH of meat to be used for freezer-storage could solve most of the problems involved in such processing. As already indicated, it is possible to tailor meat to the desired properties by pre-slaughter treatment. Much improvement might be attained in freezer-stored meat by selection of fresh meat with pH in the upper range. According to the knowledge already available but incompletely tested, there should be no great difficulty in adjusting the ultimate pH to the desired level. Summarizing all information on the effect of different kinds of stress on animals before slaughter, evidently a high ultimate pH can be attained with little adverse effect on palatability. The following important characteristics are obtained. The muscle tenderness is increased, juiciness is higher, and color is more stable, although somewhat darker. Even at relatively slow rates of freezing, drip is small due to the greater waterholding capacity and stability of the proteins. There has been some disagreement in reports with regard to meat flavor. Some findings have indicated that a high ultimate pH results in decreased flavor. Other workers have detected no differences in flavor. The consensus appears to indicate little significant change in flavor. Such meat is more susceptible to bacterial attack, and adsorbs curing salts less rapidly. This appears to be the greatest fault of meat with a high ultimate pH. The color of the meat is darker, but it is far more stable and in any case color darkens upon freezing. The desired conditions for pre-slaughter adjustment may be obtained by administration of adrenaline, neopyrithiamine, epinephrine, or sodium iodoacetate. Freezing soon after slaughter slows down the rate of ATP disappearance. One pertinent recommendation for tailoring of meat to desired properties for freezer storage is the injection of magnesium sulfate before slaughter as a relaxant to slow down the subsequent rate of decrease of ATP, followed by freezing post-mortem in the course of five hours.

Control of meat properties according to the various means discussed may seem not practical at this time. This is to some extent true, and certainly more study is needed of such processes. However, since it is probable that most meat in the future will be marketed as prepackaged units, and since a large part of the meat may be freezer-stored until purchased, uniformity of the meat supply will be essential. The adjustments possible by the antemortem and postmortem treatments will tend to improve stability and color,

increase the effectiveness of antioxidants, and give better overall palatability.

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² Space did not permit citing all applicable references. An attempt has been made, for the most part, to list only recent papers and reviews. These are deemed a sufficient basis for further study of the subjects discussed.

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